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拟南芥叶片衰老过程中细胞溶血磷脂和膜脂不饱和度的变化*

贾艳霞[1,2], 李唯奇[1**]
(1 中国科学院昆明植物研究所 中国西南野生生物种质资源库, 云南 昆明 650201;
2 中国科学院大学, 北京 100049)

摘要: 细胞膜的流动性和渗透性的改变是植物衰老过程中一个内在的、具有破坏性的变化。膜脂组成中, 溶血磷脂的出现是膜伤害的一个重要标志; 膜脂双键数目的变化是影响膜流动性的主要因素。应用脂类组学的方法, 检测了拟南芥野生型及其磷脂酶 Dδ (PLDδ) 缺失型突变体在离体诱导的、脱落酸 (abscisic acid, ABA) 和乙烯 (ethylene) 促进的衰老过程中, 溶血磷脂 (lysophospholipids, lysoPLs) 的分子变化, 并通过计算膜脂双键指数 (double bond index, DBI) 表征了膜流动性的变化。结果表明, 在离体诱导的衰老过程和乙烯促进的衰老过程中, 溶血磷脂的总含量和各溶血磷脂分子的变化不显著, 而在 ABA 促进的衰老过程中溶血磷脂总含量和部分溶血磷脂分子均显著升高; 在上述三种衰老处理下, 总膜脂的 DBI 均下降, 但是离体诱导和激素促进的的衰老过程中各类膜脂的 DBI 的变化却不同。同时我们还发现, 抑制 PLDδ 基因表达降低了 ABA 促进的衰老过程中溶血磷脂的产生、减缓了 ABA 和乙烯促进的衰老过程中总的膜脂的 DBI 的降低。

关键词: 拟南芥; 磷脂酶 Dδ; 乙烯; 脱落酸; 衰老
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Changes in Lysophospholipid and Degree of Unsaturated Membrane Lipids are Associated With Senescence in *Arabidopsis* Leaves

JIA Yan-Xia[1,2], LI Wei-Qi[1**]
(1 *Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650201, China; 2 *University of Chinese Academy of Sciences*, Beijing 100049, China)

Abstract: Alterations of membrane fluidity and permeability are an inherent feature of deterioration associated with senescence in plants. With regard to membrane lipid constituents, the occurrence of lysophospholipid (lysoPL) is a signal of membrane damage, while changes in the double bond number of membrane lipids has important effects on membrane fluidity. In the present study, a lipidomics approach was used to study changes in lysoPL molecular species, and, in addition, the double bond index (DBI) of membrane lipids was calculated to indicate membrane fluidity in wild type (WS) and a phospholipid Dδ (PLDδ) knockout mutant of *Arabidopsis* during detachment-induced, abscisic acid (ABA)- or ethylene-promoted senescence. The results indicated that the content of total lysoPLs and some lysoPL molecular species increased markedly during ABA-promoted senescence, while no significant change was detected during detachment-induced and ethylene-promoted senescence. The DBI of total membrane lipids decreased during three senescence treatments; however, the pattern of change for each membrane lipid class differed between detachment-induced and hormone-promoted senescence. Suppression of PLDδ attenuated lysoPLs accumula-

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** Author for correspondence; E-mail: weiqili@mail.kib.ac.cn

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作者简介: 贾艳霞 (1982-) 女, 博士, 工程师, 主要从事植物逆境分子生理学研究。E-mail: jiayanxia@mail.kib.ac.cn

tion during ABA-promoted senescence, and slowed down the decrease of DBI of the total membrane lipids during ABA- and ethylene-promoted senescence.

Key words: *Arabidopsis*; Phosphalipase Dδ; Ethylene; ABA; Senescence

Abbreviations: abscisic acid (ABA); phospholipase D (PLD); Lysophospholipid (lysoPL); double bond index (DBI); phosphatidylcholine (PC); phosphatidylglycerol (PG); phosphatidylinositol (PI); phosphatidic acid (PA); phosphatidylserine (PS); digalactosyldiacylglycerol (DGDG); monogalactosyldiacylglycerol (MGDG); phosphatidylethanolamine (PE); electrospray ionization tandem mass spectrometry (ESI-MS/MS)

Leaf senescence, as an integral part of plant development, occurs in the last stage of leaf development (Lim *et al.*, 2007). The changes most closely associated with senescence are declines in total protein and RNA levels and chloroplast breakdown. Under constant environmental conditions, senescence occurs in response to aging and is relatively constant and predictable (Hensel *et al.*, 1993). However, certain stress such as drought or nutrient limitation or darkness can result in premature senescence, shortening the lifetime of individual leaves or indeed the whole plants. Moreover, hormones also are able to hasten or repress senescence, and ABA and ethylene was known for accelerating leaf senescence and inducing visible yellowing (Oh *et al.*, 1996; Weaver *et al.*, 1998).

Macromolecule degradation is likely to increase the levels of radical species such as reactive oxygen species (ROS) in senescing tissues (Thompson *et al.*, 1998). The increased of radical species can induce lipid peroxidation, which alter membrane fluidity and permeability (Thompson *et al.*, 2000). Membrane deterioration leading to leakiness and loss of selective permeability is an early and ubiquitous feature of senescence (Fan *et al.*, 1997; Lim *et al.*, 2005; Espinoza *et al.*, 2007; Martínez *et al.*, 2008). Membrane lipids (Wanner *et al.*, 1991), play a pivotal role by impacting membrane properties like fluidity, permeability and active transport. Generally, the unsaturation degree of membrane lipids is used to reflect membrane fluidity (Bakht *et al.*, 2006). Galactolipids (monogalactosyldiacylglycerol, MGDG; digalactosyldiacylglycerol, DGDG), which are the dominant component of thylakoid membranes, are main contributors to membrane unsaturation because they harbor more trienoic fatty acids than other membrane phospholipids (phosphatidylcholine, PC; phosphatidylethanolamine, PE). Disruption of membrane integrity is additional important feature associated with senescence in plant (Fan *et al.*, 1997; Thompson *et al.*, 1998). The levels of total lipids decrease in senescing leaves and it appears that the membranes of the cell including the thylakoid membranes are metabolized to provide energy for senescence process (Harwood *et al.*, 1982; Wanner *et al.*, 1991). Lysophospholipids (LysoPLs) including lysophosphatidylcholine (lysoPC), lysophosphatidylglycerol (lysoPG), and lysophosphatidylethanolamine (lysoPE), are derived from the hydrolysis of phospholipids at the *sn-1* or *sn-2* position of the glycerol backbone. In comparison with other membrane glycerolipid, the content of lysoPLs in *Arabidopsis* is low but very sensitive to stress such as freezing, heat-shock, and dehydration. Upon exposure to stress, lysoPLs usually increase by five- to 20- fold within hour or even minutes (Welti *et al.*, 2002; Li *et al.*, 2008). Exogenous application of lysoPE is purported to delay leaf senescence in plants (Hong *et al.*, 2009). However, how the endogenous lysoPL response to leaf senescence is unknown.

Phospholipase Dδ (PLDδ), which is one of twelve PLDs in *Arabidopsis*, has several properties that distinguish it from other PLDs (Gardiner *et al.*, 2001; Wang and Wang, 2001). PLDδ is activated by oleic acid and is tightly associated with the plasma membrane and microtubule cytoskeleton (Gardiner *et al.*, 2001; Wang and Wang, 2001). Analy-

ses of PLDδ-altered *Arabidopsis* suggest that PLDδ positively regulate plant tolerance to stress such as freezing (Li *et al.*, 2004; Li *et al.*, 2008), and ultra-violet irradiation (Zhang *et al.*, 2003). According to our previous study, suppression of PLDδ retarded ABA-and ethylene-promoted senescence (Jia *et al.*, 2013), while the change in lysoPLs content, the unsaturation degree of membrane glycerolipids and the effects of PLDδ during such senescence process have not been investigated.

In the present study, electrospray ionization and tandem mass spectrometry (ESI-MS/MS) was used to examine the changes in the lysoPLs and the unsaturation degree of membrane lipid during detachment-induced leaf senescence and ABA- or ethylene-promoted leaf senescence in wild type (WS) and PLDδ-KO mutant *Arabidopsis*. The results showed that response patterns of lysoPLs were different, while changes in DBI of membrane lipid were similar during ABA- and ethylene-promoted senescence. Moreover, the association of lysoPLs and DBI of membrane lipid with PLDδ-mediated retardation of ABA- and ethylene-promoted senescence were also been analyzed.

1 Methods and materials

1.1 Plant materials

A PLDδ-kncokout mutant was preciously isolated from *Arabidopsis* Wassilewskija ecotype (WS). The loss of PLDδ was confirmed by the absence of the transcript, protein, and activity of PLDδ (Li *et al.*, 2008).

1.2 Growth conditions and hormone treatments

Two *Arabidopsis* genotypes were hydroponic system in a controlled growth chamber with 23 ℃ (day) and 19 ℃ (night) and 60% relative humidity under a 12-h photoperiod fluorescent lighting of 120 μmol $\cdot m^{-2} \cdot s^{-1}$. Fully expanded leaves were collected from 6-week-old *Arabidopsis* plants. The detached leaves were rinsed briefly with sterile water and placed adaxial side up in Petri dishes containing water or the final concentration of 50 μM ABA (Sigma-Aldrich, St Louis, USA, A1049) or ethephon (Sigma-Aldrich, St Louis, USA, C0143). Ethephon was chosen to treat the detached leaves, because it was easier to control and have identical effect on the detached leaves comparing with ethylene. The leaves were incubated at ± 23 ℃ under a 12-h photoperiod and light of 120 μmol $\cdot m^{-2} \cdot sec^{-1}$.

1.3 Lipid extraction and ESI/MS-MS analysis

The process of lipid extraction, ESI-MS/MS analysis, and quantification was performed as described previously with minor modification (Welti *et al.*, 2002; Li *et al.*, 2008). Each sample contained two or three detached leaves with a pooled dry weight of 2 to 9 mg, and five replicates for each genotype were analyzed. To inhibit lipolytic activities, leaves were transferred immediately into 3 mL of isopropanol with 0.01% butylated hydroxytoluene at 75 ℃ and extracted several times with chloroform/methanol (2∶1) with 0.01% butylated hydroxytoluene, until all of the remaining leaves appeared white. Automated ESI-MS/MS analysis was performed in the Kansas Lipidomics Research Center Analytical Laboratory.

1.4 Data analysis

Statistical analysis was performed using Origin 7.0 (OriginLab Corporation, Northampton, MA, USA). Double bond index (DBI) were calculated with the formula: $DBI = [\sum(N \times mol\%\ lipid)]/100$, where N was the total number of double bonds in the two fatty acid chains of each glycerolipid molecule (Zheng *et al.*, 2011). For all quantitative measurements in present study, five replicates from each sampling time were analyzed. The data were subjected to one-way analysis of variance (ANOVA) with SPSS 16.0. Statistical significance was tested by Fisher's least significant difference (LSD) method.

2 Results and Discussion

2.1 Total lysoPLs increased significantly while PLDδ-KO mutant *Arabidopsis* showed lower lysoPLs during ABA-promoted senescence

That fact that exogenous application of lysoPE delay leaf senescence (Hong *et al.*, 2009) urged us

to find out how the endogenous lysoPLs (lysoPE, lysoPC and lysoPG) respond to three treatments that induced senescence artificially. In the first, detachment-induced senescence, detached *Arabidopsis* leaves were floated on water. In the second and the third, detachment-induced senescence was accelerated by the addition of ABA and ethephon, respectively.

During ABA-promoted senescence, the content of lysoPC, lysoPE and lysoPG increased significantly, which increase by 180%, 250% and 200% of initial level at day 5, respectively (Table 1). Leaves treated with water or ethylene did not show significant changes in total lysoPLs content comparing with ABA treatment (Table 1). Moreover, losing PLDδ activity attenuated amount of accumulated lysoPLs compared to WS during ABA-promoted senescence, but not during detachment-induced and ethylene-promoted senescence (Table 1). These results suggested that endogenous lysoPLs only increased during ABA-promoted senescence, whereas no significant changes were detected during detachment-induced or ethylene-promoted senescence, and the change of lysoPLs might not be a universal process (such as chlorophyll content) during leaf senescence. Thus, more evidences were needed to verify the effect of endogenous lysoPLs on leaf senescence.

2.2 The change patterns of lysoPLs molecular species were different in the three senescence treatments

The change patterns of each lysoPL molecular species responding to senescence were also be analyzed. LysoPLs contain mostly 16:0, 18:2, and 18:3 species, although 16:1 is also a significant molecular species in lysoPE. When leaves incubated in water, no significant changes in lysoPLs species were discovered, except for some individual molecular species, such as 16:0-, 18:2-, 18:3-lysoPE (Fig. 1A). During ABA-promoted senescence, unsaturated lysoPL molecular species, such as 18:3-lysoPC, 16:1-, 18:3-, and 18:2-lysoPE, and saturated molecular species, such as 16:0-LysoPC and 16:0-lysoPE tended

Table 1 Changes in total amount of lysoPLs classes under three senescence treatments.
The dry weight is dry weight minus lipid

Lipids classes	Treatments	Genotypes	Lipids (dry weight)/nmol·(mg)$^{-1}$			RC/%	
			0 day	3 days	5 days	3 days	5 days
LysoPG	water	WS	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.00^{a}	—	—
		PLDδ-KO	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.00^{a}	—	—
	ABA	WS	0.02 ± 0.01^{b}	0.02 ± 0.00^{b}	0.06 ± 0.03^{a}	—	200
		PLDδ-KO	0.02 ± 0.01^{a}	0.01 ± 0.01^{a*}	0.01 ± 0.00^{a*}	—	—
	ETH	WS	0.02 ± 0.01^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	—	—
		PLDδ-KO	0.02 ± 0.01^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	—	—
LysoPC	water	WS	0.05 ± 0.02^{a}	0.02 ± 0.01^{b}	0.04 ± 0.01^{a}	-60	—
		PLDδ-KO	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	—	—
	ABA	WS	0.05 ± 0.02^{b}	0.12 ± 0.02^{a}	0.14 ± 0.01^{a}	140	180
		PLDδ-KO	0.05 ± 0.01^{c}	0.08 ± 0.02^{b*}	0.12 ± 0.03^{a}	60	140
	ETH	WS	0.05 ± 0.02^{a}	0.06 ± 0.02^{a}	0.04 ± 0.01^{a}	—	—
		PLDδ-KO	0.05 ± 0.01^{a}	0.04 ± 0.02^{a}	0.04 ± 0.01^{a}	—	—
LysoPE	water	WS	0.04 ± 0.01^{a}	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	-50	—
		PLDδ-KO	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.03 ± 0.02^{a}	—	—
	ABA	WS	0.04 ± 0.01^{c}	0.10 ± 0.01^{b}	0.14 ± 0.02^{a}	150	250
		PLDδ-KO	0.04 ± 0.01^{c}	0.08 ± 0.01^{b*}	0.11 ± 0.02^{a*}	100	175
	ETH	WS	0.04 ± 0.01^{a}	0.06 ± 0.03^{a}	0.06 ± 0.03^{a}	—	—
		PLDδ-KO	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	—	—

Values are means ±*S. D.* (n=4 or 5). Values with different letters are significantly different (P<0.05). "*" indicates that the value is significantly different from that of the WS under the same condition (P<0.05)

to increase, while there was no significant change in other lysoPL molecular species during ABA-promoted senescence (Fig. 1B). Although ethylene also promoted leaf senescence, the patterns of lysoPLs molecular species change in ethylene-promoted senescence was different from that in ABA-promoted senescence. During ethylene-promoted senescence, the content of 18∶2-lysoPC and 18∶2-lysoPE decreased to 33% and 50% of initial levels, respectively (Fig. 1C). Ablation of PLDδ resulted in less increasing of 16∶0-, 18∶3-lysoPC and 16∶1-lysoPE molecular species during ABA-promoted senescence comparing with WS, while no differences in levels of any lysoPL species were detected between WS and PLDδ-KO plants during detachment-induced and ethylene-promoted senescence (Fig. 1). The results suggested that ABA and ethylene both promoted leaf senescence while differed in lysoPLs molecular species response. PLDδ only involved in some lysoPLs molecular species production during ABA-promoted senescence, but more studies were needed to prove if it contributed to the retardation of ABA-promoted senescence.

2.3 The change patterns of DBI were different among lipid classes

The unsaturation degree of membrane lipid significantly impacts the fluidity of the membranes. DBI was employed to indicate the unsaturation degree of membrane glycerolipids. The DBI is the average number of double molecular species and a high DBI indicates the presence of more unsaturated membrane lipids, and vice versa.

The DBI of plastidic lipids MGDG was 5.9, which was much higher comparing to other lipid classes, while the DBI of phosphatidylserine (PS) was lower than other lipids, which was only 2.5. The change of DBI in each membrane lipid was different between detachment-induced and hormone-promoted senescence. During detachment-induced senescence, the DBI of four kinds of lipids (MGDG, DGDG, PG, PC) decreased, while the DBI of other lipids remained unchanged (Table 2). When

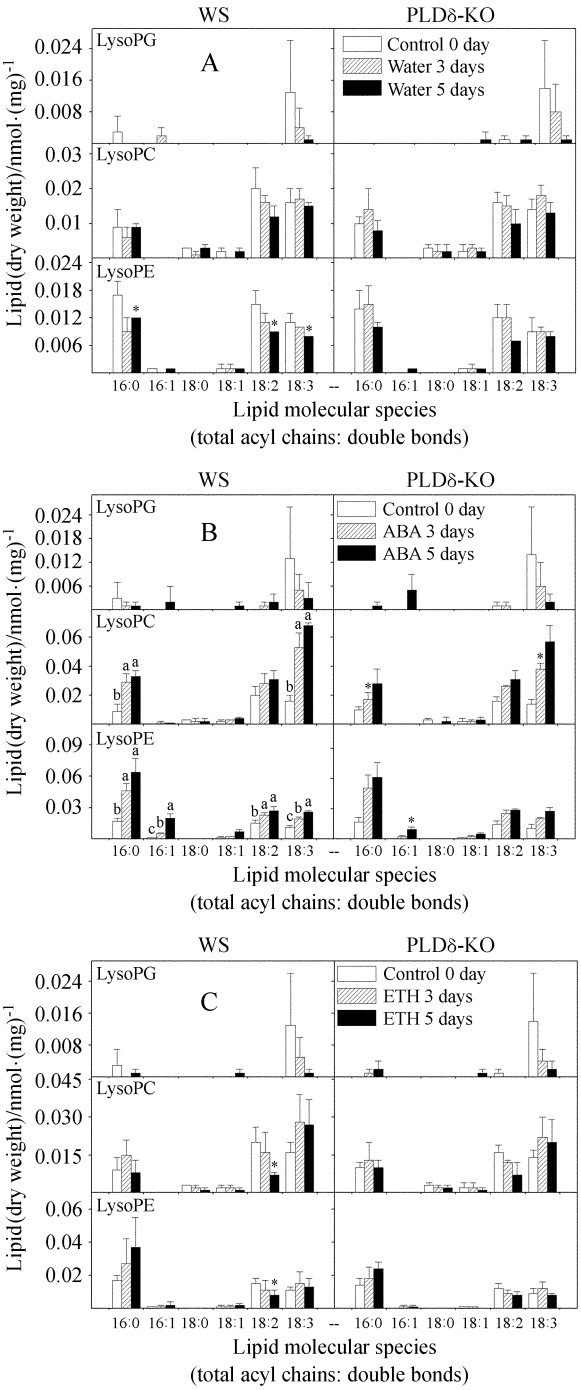

Fig. 1　Changes in lysoPL molecular species in *Arabidopsis* as revealed by ESI-MS/MS under three senescence treatments. Left panel, lipid of wild type WS plant; right panel, lipid of PLDδ-knockout mutant plant. The white bars represent nontreated leaves, and the double-hatched bars represent treated for 3 days leaves, and the black bars represent for 5 days leaves. The values are the means ±*SD*. (n=4 or 5) A. The change of lysoPL molecular species during detachment-induced senescence; B. The change of lysoPL molecular species during ABA-promoted senescence; C. The change of lysoPL molecular species during ethylene-promoted senescence

Table 2 Changes in double bond index (DBI) of each lipid classes under three senescence treatments

Lipids classes	Treatments	Genotypes	Double bond index (DBI)			RC/%	
			0 day	3 days	5 days	3 days	5 days
PG	water	WS	3.41 ± 0.02^{a}	3.38 ± 0.07^{a}	3.00 ± 0.07^{b}	—	−12.02
		PLDδ-KO	3.43 ± 0.08^{a}	3.38 ± 0.07^{a}	3.06 ± 0.14^{b}	—	−10.79
	ABA	WS	3.41 ± 0.02^{a}	3.23 ± 0.17^{b}	2.89 ± 0.14^{c}	−5.28	−15.25
		PLDδ-KO	3.43 ± 0.08^{a}	3.27 ± 0.06^{b}	3.05 ± 0.10^{c}	−4.66	−11.08
	ETH	WS	3.41 ± 0.02^{a}	3.39 ± 0.11^{ab}	3.26 ± 0.11^{b}	—	−4.40
		PLDδ-KO	3.43 ± 0.08^{a}	3.23 ± 0.18^{b}	3.38 ± 0.05^{ab}	−5.83	—
PC	water	WS	3.86 ± 0.05^{a}	3.63 ± 0.06^{c}	3.71 ± 0.04^{b}	−5.96	−3.89
		PLDδ-KO	3.88 ± 0.03^{a}	3.66 ± 0.07^{b}	3.71 ± 0.02^{b}	−5.67	−4.38
	ABA	WS	3.86 ± 0.05^{b}	4.11 ± 0.05^{a}	4.11 ± 0.02^{a}	6.48	6.48
		PLDδ-KO	3.88 ± 0.03^{b}	4.22 ± 0.32^{a}	4.09 ± 0.02^{a}	8.76	5.41
	ETH	WS	3.86 ± 0.05^{c}	4.04 ± 0.10^{b}	4.30 ± 0.10^{a}	4.66	11.40
		PLDδ-KO	3.88 ± 0.03^{c}	3.98 ± 0.08^{b}	4.22 ± 0.08^{a}	2.58	8.76
PE	water	WS	3.26 ± 0.03^{a}	3.25 ± 0.02^{a}	3.27 ± 0.03^{a}	—	—
		PLDδ-KO	3.28 ± 0.02^{a}	3.27 ± 0.04^{a}	3.30 ± 0.01^{a}	—	—
	ABA	WS	3.26 ± 0.03^{c}	3.41 ± 0.02^{a}	3.35 ± 0.02^{b}	4.60	2.76
		PLDδ-KO	3.28 ± 0.02^{b}	3.38 ± 0.02^{a}	3.37 ± 0.02^{a}	3.05	2.74
	ETH	WS	3.26 ± 0.03^{b}	3.53 ± 0.06^{a}	3.60 ± 0.07^{a}	8.28	10.43
		PLDδ-KO	3.28 ± 0.02^{b}	3.54 ± 0.07^{a}	3.53 ± 0.05^{a*}	7.93	7.62
PI	water	WS	2.74 ± 0.06^{a}	2.69 ± 0.05^{a}	2.70 ± 0.05^{a}	—	—
		PLDδ-KO	2.71 ± 0.04^{a}	2.70 ± 0.04^{a}	2.71 ± 0.04^{a}	—	—
	ABA	WS	2.74 ± 0.06^{b}	2.79 ± 0.02^{b}	2.83 ± 0.03^{a}		3.28
		PLDδ-KO	2.71 ± 0.04^{b}	2.77 ± 0.03^{a}	2.77 ± 0.05^{a}	2.21	2.21
	ETH	WS	2.74 ± 0.06^{b}	2.83 ± 0.04^{a}	2.92 ± 0.08^{a}	3.28	6.57
		PLDδ-KO	2.71 ± 0.04^{b}	2.85 ± 0.07^{a}	2.87 ± 0.05^{a}	5.17	5.90
PA	water	WS	3.92 ± 0.80^{a}	3.34 ± 0.83^{b}	3.93 ± 0.49^{a}	−14.79	—
		PLDδ-KO	3.57 ± 1.12^{a}	3.19 ± 0.90^{b}	3.74 ± 0.90^{a}	−10.64	—
	ABA	WS	3.92 ± 0.80^{a}	3.36 ± 0.36^{a}	3.53 ± 0.39^{a}	—	—
		PLDδ-KO	3.57 ± 1.12^{a}	3.09 ± 0.20^{a}	3.43 ± 0.23^{a}	—	—
	ETH	WS	3.92 ± 0.80^{a}	3.88 ± 0.89^{a}	3.10 ± 0.82^{a}	—	—
		PLDδ-KO	3.57 ± 1.12^{a}	3.49 ± 0.56^{a}	3.74 ± 0.90^{a}	—	—
PS	water	WS	2.52 ± 0.18^{a}	2.49 ± 0.14^{a}	2.50 ± 0.22^{a}	—	—
		PLDδ-KO	2.60 ± 0.34^{a}	2.63 ± 0.22^{a}	2.70 ± 0.33^{a}	—	—
	ABA	WS	2.52 ± 0.18^{a}	2.76 ± 0.18^{a}	2.67 ± 0.29^{a}	—	—
		PLDδ-KO	2.60 ± 0.34^{a}	2.56 ± 0.10^{a}	2.68 ± 0.14^{a}	—	—
	ETH	WS	2.52 ± 0.18^{a}	2.49 ± 0.14^{a}	2.91 ± 0.81^{a}	—	—
		PLDδ-KO	2.60 ± 0.34^{a}	2.63 ± 0.22^{a}	2.70 ± 0.33^{a}	—	—
MGDG	water	WS	5.91 ± 0.01^{a}	5.89 ± 0.01^{b}	5.86 ± 0.01^{b}	−0.34	−0.85
		PLDδ-KO	5.91 ± 0.01^{a}	5.89 ± 0.01^{b}	5.86 ± 0.01^{b}	−0.34	−0.85
	ABA	WS	5.91 ± 0.01^{b}	5.91 ± 0.01^{b}	5.93 ± 0.01^{a}	—	0.34
		PLDδ-KO	5.91 ± 0.01^{b}	5.92 ± 0.01^{ab}	5.93 ± 0.00^{a}	—	0.34
	ETH	WS	5.91 ± 0.01^{b}	5.93 ± 0.02^{ab}	5.95 ± 0.01^{a}	—	0.68
		PLDδ-KO	5.91 ± 0.01^{b}	5.92 ± 0.02^{ab}	5.93 ± 0.01^{a*}	—	0.34
DGDG	water	WS	5.17 ± 0.02^{a}	5.15 ± 0.04^{a}	4.98 ± 0.04^{b}	—	−4.05
		PLDδ-KO	5.15 ± 0.03^{a}	5.18 ± 0.04^{a}	5.02 ± 0.05^{b}	—	−3.09
	ABA	WS	5.17 ± 0.02^{b}	5.28 ± 0.04^{ab}	5.32 ± 0.06^{a}	—	2.50
		PLDδ-KO	5.15 ± 0.03^{b}	5.32 ± 0.03^{ab}	5.32 ± 0.02^{a}	—	3.30
	ETH	WS	5.17 ± 0.02^{b}	5.36 ± 0.10^{a}	5.36 ± 0.09^{a}	3.28	3.28
		PLDδ-KO	5.15 ± 0.03^{b}	5.25 ± 0.16^{ab}	5.37 ± 0.01^{a}	—	4.27

Values are means ±*S. D* (n=4 or 5). Values with different letters are significantly different (P<0.05). "*" indicates that the value is significantly different from that of the WS under the same condition (P<0.05). DBI=(∑[N×mol % molecular specie])/100, N is the number of double bonds in each molecular specie

leaves incubated in ABA or ethylene, the DBI of five types of lipid classes [MGDG, DGDG, PC, PE and PI (phosphatidylinositol)] increased, while the DBI of PG decreased, and the DBI of PA, PS maintained. During ABA- and ethylene-promote senescence, the DBI of plastidic lipids MGDG showed the smallest changes among all lipid classes, which increased to 0.34% and 0.68% of initial levels in WS plants, respectively (Table 2). The change patters of DBI of lysoPLs were different among three senescence treatments, although the content of LysoPLs was minor compared to other membrane glycerolipids (Table 3). The DBI of the main membrane lipids (MGDG, DGDG and PC) increased during ABA- and ethylene-promoted senescence while decreased during detachment-induced senescence, the differences might be resulted from the effects of the hormone themselves on the unsaturation of the membrane lipids.

2.4 The DBI of total membrane lipids decreased during three senescence treatments

The DBI of total membrane lipids were analyzed, and the results showed that the DBI of total membrane lipids decreased significantly during three senescence treatments. However, what's interesting was that the DBI of total membrane lipids declined while the DBI of most lipid classes increased during ABA- and ethylene-promoted senescence (Tables 2–4). The decline of DBI of total membrane lipids might be caused by the dramatic degradation of plastidic lipid MGDG which occupy about 77% of the total membrane lipids (Li *et al.*, 2008). Koiwai *et al.* (1981) have reported that chloroplast lipid MGDG and DGDG degraded more rapidly than non-chloroplastic lipids PE, PC, PI and PS (mainly located in the membranes of non-photosynthetic organelles such as endoplasmic reticulum and mitochondria). The DBI was calculated based on the mol% content of each lipid molecule species, so it is conceivable that the dramatic degradation of galactolipids might contribute to the decrease of DBI of total membrane lipids.

Table 3　Changes in double bond index (DBI) of lysoPLs classes under three senescence treatments

Lipids classes	Treatments	Genotypes	Double bond index (DBI)			RC/%	
			0 day	3 days	5 days	3 days	5 days
LysoPC	water	WS	1.83 ± 0.16^{a}	1.65 ± 0.17^{a}	1.71 ± 0.19^{a}	—	—
		PLDδ-KO	1.69 ± 0.13^{a}	1.69 ± 0.18^{a}	1.79 ± 0.84^{a}	—	—
	ABA	WS	1.83 ± 0.16^{a}	1.89 ± 0.04^{a}	1.96 ± 0.06^{a}	—	—
		PLDδ-KO	1.69 ± 0.13^{b}	2.01 ± 0.13^{a}	1.96 ± 0.84^{ab}	—	—
	ETH	WS	1.83 ± 0.16^{b}	1.83 ± 0.10^{b}	2.16 ± 0.24^{a}	—	18.03
		PLDδ-KO	1.69 ± 0.13^{b}	1.85 ± 0.19^{b}	1.85 ± 0.15^{a*}	—	9.47
LysoPG	water	WS	1.46 ± 0.04^{a}	1.25 ± 0.11^{b}	1.42 ± 0.09^{a}	-14.38	—
		PLDδ-KO	1.48 ± 0.03^{a}	1.42 ± 0.09^{a}	1.50 ± 0.06^{a}	—	—
	ABA	WS	1.46 ± 0.04^{a}	1.18 ± 0.07^{b}	1.12 ± 0.12^{b}	-19.18	-23.29
		PLDδ-KO	1.48 ± 0.03^{a}	1.17 ± 0.12^{b}	1.18 ± 0.08^{b}	-20.95	-20.27
	ETH	WS	1.46 ± 0.04^{a}	1.26 ± 0.11^{b}	0.97 ± 0.09^{c}	-13.70	-33.56
		PLDδ-KO	1.48 ± 0.03^{a}	1.36 ± 0.22^{a}	0.96 ± 0.09^{b}	—	-35.14
LysoPE	water	WS	2.22 ± 0.76^{a}	2.40 ± 0.66^{a}	2.04 ± 0.88^{a}	—	—
		PLDδ-KO	2.82 ± 0.23^{a}	3.00 ± 0.00^{a}	1.86 ± 0.65^{b}	—	-34.04
	ABA	WS	2.22 ± 0.76^{a}	2.52 ± 0.51^{a}	1.71 ± 0.60^{a}	—	—
		PLDδ-KO	2.82 ± 0.23^{a}	2.48 ± 0.64^{ab}	1.70 ± 0.79^{b}	—	-39.72
	ETH	WS	2.22 ± 0.76^{a}	3.00 ± 0.00^{a}	0.99 ± 0.41^{b}	—	-55.41
		PLDδ-KO	2.82 ± 0.23^{a}	2.32 ± 0.86^{ab}	1.88 ± 0.65^{b*}	—	-33.33

Values are means ±*S. D* (n=4 or 5). Values with different letters are significantly different (P<0.05). "*" indicates that the value is significantly different from that of the WS under the same condition (P<0.05). DBI=(∑[N×mol % molecular specie])/100, N is the number of double bonds in each molecular specie

Table 4 Changes in double bond index (DBI) of total membrane lipids under three senescence treatments

Treatments	Genotypes	Double bond index (DBI)			RC/%	
		0 day	3 days	5 days	3 days	5 days
water	WS	5.53 ± 0.03[a]	5.44 ± 0.05[b]	5.34 ± 0.04[c]	−1.63	−3.43
	PLDδ-KO	5.53 ± 0.04[a]	5.46 ± 0.02[b]	5.31 ± 0.02[c]	−1.27	−3.98
ABA	WS	5.53 ± 0.03[a]	5.44 ± 0.04[b]	5.12 ± 0.01[c]	−1.63	−7.41
	PLDδ-KO	5.53 ± 0.04[a]	5.44 ± 0.05[b]	5.17 ± 0.01[c*]	−1.63	−6.51
ETH	WS	5.53 ± 0.03[a]	5.45 ± 0.05[b]	5.15 ± 0.01[c]	−1.45	−6.87
	PLDδ-KO	5.53 ± 0.04[a]	5.41 ± 0.04[b]	5.28 ± 0.01[c*]	−2.17	−4.52

Values are means ±*S. D* (n=4 or 5). Values with different letters are significantly different (P<0.05). "*" indicates that the value is significantly different from that of the WS under the same condition (P<0.05). DBI=(∑[N×mol % molecular specie])/100, N is the number of double bonds in each molecular specie

2.5 Suppression of PLDδ slowed down the decrease of DBI of total lipids, while no effect on the DBI of different lipid classes

To investigate the role of unsaturation of membrane glycerolipids in senescence, we analyzed the DBI of membrane lipids in PLDδ-KO plants, in which hormone-promoted senescence was delayed relative to that in WS plants (Tables 2−3). The DBI of total membrane lipids were identical between WS and PLDδ-KO plants during detachment-induced senescence, while which were significantly higher in PLDδ-KO leaves than in WS leaves during ABA- and ethylene-promoted senescence at day 5 (Table 4). However, no significant differences of DBI were detected in each membrane lipid class between WS and PLDδ-KO plants during three senescence treatments. The data indicated that the delayed senescence was associated with the suppression of PLDδ correlated with a decrease in DBI of total lipids, which also was contributed to the slower degradation of galactolipids in PLDδ-KO plants. Our lipid profiling indicated that suppressing PLDδ retarded ABA-promoted leaf senescence by attenuating lipid degradation, and PA level was significant difference between WS and PLDδ-KO plants (Jia *et al.*, 2013). Here showed that suppression of PLDδ did not affect DBI level of PA. This suggested that PA played a role in hormone-promoted senescence by exchanging head group with other lipids but not by changing the double bonds of its acyl groups.

In conclusion, our results suggested that the patterns of lysoPL changes were not completely identical, while the unsaturation degree of membrane lipids was similar in ABA- to in ethylene-promoted senescence. Suppression of PLDδ attenuated 16∶0- and 18∶3-lysoPC species and 16∶1-lysoPE species production during ABA-promoted senescence and also slowed down the decrease of DBI of total membrane lipids during ABA- and ethylene-promoted senescence. However, many questions still remained unclear and would be further studied in future, such as metabolism of different lipid profiles in ABA- and ethylene-promoted leaf senescence, the mechanism of suppression of PLDδ delayed ABA- and ethylene-promoted senescence, and the effect of the endogenous lysoPLs and the unsaturation degree of membrane lipids on leaf senescence.

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